

IN THE CLAIMS:

Claim 1 (previously presented) A recombinant nucleotide sequence of expression cassette OXY-1 of SEQ ID No. 1.

Claim 2 (currently amended) A recombinant nucleotide sequence that encodes staphylokinase SAK-2 ~~gene~~ protein, the nucleotide sequence comprising nucleotides 13-409 of SEQ ID No. 2.

Claim 3 (currently amended/withdrawn) An amino acid peptide sequence of recombinant staphylokinase SAK-2 ~~gene~~ protein of SEQ ID No. 3.

Claim 4 (currently amended) A plasmid as claimed in claim 28, wherein the plasmid is plasmid pRM1 contained in *E. coli* having of International Deposition No. ~~BPL-0019~~ 5146 in the "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India.

Claim 5 (currently amended) A plasmid pOXYSK-1 contained in *E. coli* having of International Deposition No. ~~BPL-0020~~ 5147 in the "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India.

Claim 6 (currently amended) A plasmid pOXYSK-2 contained in *E. coli* having of International Deposition No. ~~BPL-0021~~ 5148 in the "Microbial Type Culture Collection"

at Institute of Microbial Technology, Chandigarh, India.

Claim 7 (currently amended) A recombinant *E. coli* of International Deposition No. 5146, ~~the International Depository is in the~~ "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, ~~having a plasmid pRM1 of International Deposition No. BPL-0019.~~

Claim 8 (currently amended) A recombinant *E. coli* of International Deposition No. 5147, deposited in the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, ~~having a plasmid pOXYSAK-1 of International Deposition No. BPL-0020.~~

Claim 9 (currently amended) A recombinant *E. coli* of International Deposition No. 5148, deposited in the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, ~~having a plasmid pOXYSAK-2 of International Deposition No. BPL-0021.~~

Claim 10 (withdrawn) A process for over-producing staphylokinase and its analogues by modulating oxygen level, said method comprising the steps of:

- a. isolating a staphylokinase gene encoding an SAK protein,
- b. modifying codons in the gene encoding Lys6 and Lys8 residues present at an amino-terminal end of the SAK protein to obtain a modified DNA,

- c. providing the recombinant nucleotide sequence of expression cassette OXY-1 as claimed in claim 1,
- d. integrating the modified DNA obtained in step (b) with the recombinant nucleotide sequence of expression cassette OXY-1 to obtain a product comprising pOXYPRO,
- e. integrating the product obtained in step d with plasmid vector PRM 1 to obtain plasmid constructs pOXYSAK-1, and pOXYSAK-2 respectively,
- f. introducing the plasmid constructs of step (e) into a host system,
- g. culturing the host cell for over-production of SAK or its derivatives under high aeration and changing level of oxygen below 5% of atmospheric oxygen level when cell growth reaches to exponential phase to obtain cell mass,
- h. lysing the of the cell mass cells of step (g) to separate cell lysate from cellular debris, and thereby obtaining the staphylokinase and its analogues.

Claim 11 (withdrawn) A process as claimed in claim 10, wherein the Lys6 and Lys8 residues of the SAK protein are changed into small and neutral amino acid residues.

Claim 12 (withdrawn) A process as claimed in claim 10, wherein the plasmid vector is a high or medium copy number plasmid.

Claim 13 (withdrawn) A process as claimed in claim 10, wherein the host system is selected from the group consisting of *E. coli*, *Bacillus*, and Yeast.

Claim 14 (withdrawn) A process as claimed in claim 10, wherein the sequence of OXY-1 is modified depending upon the host system.

Claim 15 (withdrawn) A process as claimed in claim 11 10, wherein the amino acid residues are selected from the group consisting of Alanine, and Glycine.

Claim 16 (withdrawn/currently amended) A process as claimed in claim 10, wherein the culturing is in growth medium is comprising Luria Broth (LB) medium.

Claim 17 (withdrawn/currently amended) A process as claimed in claim 10, ~~wherein~~ comprising culturing the host cell for over-production of SAK or its derivatives at shake flask culture or at fermentation.

Claim 18 (withdrawn) A process as claimed in claim 17, comprising culturing the host cell till O.D.<sub>600</sub> reaches 0.6 to 0.7.

Claim 19 (withdrawn) A process as claimed in claim 17, wherein the fermentation is a two-stage fed-batch fermentation.

Claim 20 (withdrawn) A process as claimed in claim 10, comprising obtaining the cell mass by centrifugation or filtration.

Claim 21 (withdrawn/currently amended) A process as claimed in claim 10, wherein the lysing of the cells is by a method selected from the group consisting of sonication, chemical, and ~~mechanical~~ mechanics lysis.

Claim 22 (withdrawn) A process as claimed in claim 10, comprising separating the cell lysate from the cellular debris by centrifugation.

Claim 23 (withdrawn/currently amended) A method of dissolving a blood clot in a subject in need thereof, said method comprising the step of administering a pharmaceutically effective amount of streptokinase analogue SAK-2, optionally along with additive(s).

Claim 24 (withdrawn) A method as claimed in claim 23, wherein the additive is selected from a group comprising nutrients consisting of proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste, and/or pharmaceutically acceptable carrier, excipient, diluent, or solvent.

Claim 25 (withdrawn) A method as claimed in claim 23, wherein the SAK-2 and additives are in a ratio ranging between 1:10 to 10:1.

Claim 26 (new). A recombinant nucleotide sequence of claim 2, wherein the nucleotide sequence comprises nucleotides 1-582 of SEQ ID NO: 2.

Claim 27 (new). A recombinant nucleotide sequence of claim 26 consisting of nucleotides

1-582 of SEQ ID NO: 2.

Claim 28 (new). A plasmid comprising the recombinant nucleotide sequence of claim 1.